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ORIGINAL ARTICLE

**Saskia Kley · Peter Tschudi · André Busato**
**Frédéric Gaschen**

# Establishing canine clinical chemistry reference values for the Hitachi® 912 using the International Federation of Clinical Chemistry (IFCC) recommendations

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**Abstract** The aim of this study was to establish population-based canine clinical chemistry reference values for the Hitachi 912 (Roche Diagnostics GmbH, Germany) with regard to age, sex, breed, housing and intended use. Reference biochemistry values for 22 variables are presented from 308 clinical healthy dogs, 145 females and 163 males, approximately 1 month to 13 years of age and of various breeds. For each variable the data were examined for homogeneity and, when suspected, outliers were excluded using the range test. Non-parametric analysis was used to calculate the conventional central 95% interval. Then the two-sided non-parametric 0.9 confidence interval of each percentile was determined. Finally, the effects of subgrouping were examined using the Kruskal–Wallis test and $p < 0.05$ was considered significant. Significant age influences were found for 20 of the 22 variables, 14 showing clinical relevance. Significant breed effects could be found for 10 of the 22 parameters, including clinically relevant lower total protein concentrations for retrievers, lower lipase activity for sled dogs, lower total bilirubin concentrations for terriers and higher total bilirubin for Molossians. Differences between male and female were present for six of the 22 variables but had no clinical relevance. Housing and intended use influenced some of the values, but these differences were of no clinical significance. We successfully established canine clinical chemistry reference values for the Hitachi 912. The IFCC Recommendations on Reference Values offered a good framework for establishing standardised reference values, and make it possible for several laboratories to share the same values. Our results clearly indicate that subgrouping according to age and breed is necessary to obtain accurate reference values.

**Keywords** Biochemistry reference values · Blood · Clinical pathology · Dog · Hitachi® 912 · IFCC

**Abbreviations** *ALAT* alanine aminotransferase · *AP* alkaline phosphatase · *ASAT* aspartate aminotransferase · *CK* creatine kinase · *GGT*γ-glutamyl transferase · *GLDH* glutamate dehydrogenase · *TP* total protein

## Introduction

Every day blood reference values are used to evaluate and compare results obtained from clinical patients. Determination of biochemical laboratory parameters allows an assessment of many of the body's metabolic and physiological processes. Variations from these reference values often provide helpful diagnostic information.

The determination of a reference range for biochemical laboratory parameters can be a challenge, owing to variations in several factors, such as the methods used and the individuals chosen as a sample of the clinically healthy reference population. Although methodology does not influence the results for many organic substances as well as electrolytes, in most cases it will cause variations in the measurement of enzyme activity. Moreover, individual factors such as breed, age and sex, and environmental factors such as season and climate, as well as physiological processes, may all affect clinically 'normal' individuals included in the reference population and cause significant variations in the reference values (Henry and Reed 1974).

Because it is impossible to establish reliable reference values for each and every individual, or even for each

S. Kley · P. Tschudi · A. Busato · F. Gaschen
University of Bern, Bern, Switzerland

F. Gaschen (✉)
Division of Small Animal Medicine,
Department of Clinical Veterinary Medicine,
University of Berne, Länggass-Strasse 128
CH-3001 Bern Switzerland.
E-mail: frederic.gaschen@kkh.unibe.ch

*Present address*: S. Kley
Clinic of Small Animal Internal Medicine, University of Zurich,
Winterthurerstrasse 260, 8057 Zurich, Switzerland

laboratory, 'standardised' population-based reference values are very valuable as they can be used to assess results obtained from different laboratories.

In 1970 the International Federation of Clinical Chemistry (IFCC) created an Expert Panel on the Theory of Reference Values. This panel published six recommendations aiming at developing a nomenclature and recommending procedures for the production, treatment and presentation of reference values. Aspects such as nomenclature of reference values, selection of reference individuals, standardised collection of specimens, analytical methods, statistical methods, and presentation of observed values in relation to reference values were all discussed in detail and resulted in six cornerstone publications (Solberg 1987; PetitClerc and Wilding 1984; Solberg and PetitClerc 1988; Solberg and Stamm 1991; Solberg 1983; Dybkaer 1982).

The aim of our study was to use the IFCC recommendations to establish population-based, univariate biochemical reference values for canine serum samples analysed with the Hitachi® 912. We also wanted to investigate clinically relevant differences between various groups of dogs according to breed, age, sex, housing and intended use.

## Materials and methods

### Animals and blood samples

Blood samples were collected over a 9-month period from 308 apparently clinically healthy dogs. Eighty-four per cent of these animals were patients of the University of Bern Small Animal Hospital. They included vaccination patients; blood donors; dogs presented for control of their ascendance; dogs undergoing screening examinations for hip and elbow dysplasia, hearing tests, or benign elective surgical interventions; and dogs belonging to students and staff. The remaining dogs were animals from humane society kennels or private family pets. Prior to blood sampling a detailed history was taken from all of the animals' owners. Questions included vaccination status, treatment against internal parasites, visits to southern Europe, current drug therapies, time of last feeding, and reproductive status. Food and water intake, urination, defecation, or the presence of exercise intolerance, coughing or vomiting were assessed. Age, sex, weight, housing type, intended use and breed type were documented. All dogs underwent a physical examination. Criteria for inclusion in the study were lack of evidence of any kind of disease (based on history and physical examination), a fasting period of about 12 h, and regular vaccination schedules as well as treatment against internal parasites. Exclusion criteria included any type of medication in the previous 2 weeks, visits to southern Europe in the previous 4 weeks, obesity, and pregnancy. Very nervous or stressed dogs were also excluded from the study.

In all cases blood was collected from the cephalic vein and directly stored in sodium fluoride and serum tubes. Serum tubes were kept at room temperature in order to allow clot formation before they were centrifuged for 10 min at 1300 *g*. The sodium fluoride tubes were centrifuged immediately for 10 min at 1300 *g*. A direct analysis of glucose from the plasma and biochemical parameters from the serum was performed.

### Analytical procedures and methods

Serum clinical determinations were measured using the Hitachi 912 automatic analyser (Roche Diagnostic GmbH, Mannheim, Germany) with reagents from Boehringer Mannheim (Mannheim, Germany). The calibration verification and quality control of the Hitachi 912 was performed daily according to the manufacturer's guidelines (Calibrator for automated systems, Precinorm U, Precipath U, ISE Standard High and Low; Roche Diagnostics GmbH, Mannheim, Germany). Estimated values and the analytical methods used are shown in Table 1. Enzyme activities were measured according to the recommendations of the IFCC at 37°C. In total, 22 parameters were determined.

### Distribution and grouping of the population

The median weight of the population was 29.4 kg (range 2.3–75.0 kg). The median age of the selected reference population was 2.42 years (range 0.1–12.9 years). Group 1 contained all dogs under 0.5 years ($n = 25$), group 2 dogs 0.5–1 year ($n = 26$), group 3 dogs 1–5

**Table 1** Test characteristics for parameters determined on the Hitachi 912

| Parameters | Methodology |
|---|---|
| Alanine aminotransferase | Modified IFCC (L-alanine and -oxoglutarate substrate) |
| Albumin | Modified Doumas (bromcresol green) |
| Alkaline phosphatase | Modified IFCC (*p*-nitrophenyl phosphate substrate) |
| Amylase | Modified IFCC (*p*-nitrophenylmaltoheptaoside substrate) |
| Aspartat aminotransferase | Modified IFCC (L-aspartate and -oxoglutarate substrate) |
| Bilirubin total | Modified Wahlefeld (DPD method) |
| Calcium | Modified Gindler (*O*-cresolphthalein complexone) |
| Chloride | Ion-selective electrode |
| Cholesterol | Enzymatic (cholesterol esterase/oxidase) |
| Creatine kinase | Modified Oliver–Rosalki (creatine phosphate substrate) |
| Creatinine | Enzymatic creatinine deiminase |
| Glucose | Enzymatic colorimetric assay (GOD–PAP) |
| γ-Glutamyl transferase | Modified IFCC (L-glutamyl-*p*-nitroanilide and glycylglycine substrate) |
| Glutamate dehydrogenase | L-oxoglutarate substrate |
| Iron | FerroZine- Methode |
| Lipase | Triolein substrate |
| Phosphorus | Modified Daly and Ertingshausen (molybdate) |
| Potassium | Ion-selective electrode |
| Protein, total serum | Modified biuret (cupric sulfate) |
| Sodium | Ion-selective electrode |
| Triglyceride | Enzymatic colorimetric assay (GPO–PAP) |
| Urea | Modified Talke and Schubert (urease) |

years ($n = 187$) and group 4 dogs over 5 years ($n = 70$). There were 101 intact females, 44 spayed females, 120 intact males and 40 neutered males. In the reference population 80.8% ($n = 249$) were different purebred dogs and 19.2% ($n = 59$) were mongrel dogs. The purebred dogs were divided into nine groups according to the 1997 guidelines of the Fédération Cynologique Internationale (International Cynologic Federation) (FCI 1997) (Table 2). A subgrouping according to the intended use of the animals was attempted, there being 223 family dogs, 61 working dogs (police dogs, guide dogs for blind people, rescue dogs) and 24 sporting dogs (agility, sled dogs, hunting dogs). Moreover, 242 dogs lived in a house or flat, whereas 66 lived in a kennel.

### Statistical analysis

Statistical analysis was performed according to the IFCC recommendations (Solberg 1983). First, a pilot study with 110 dogs was undertaken in order to determine the number of blood samples needed to estimate reference values with a 95% confidence and 10% precision. We investigated a sample size of about 311. For each variable the data were examined for homogeneity and, when suspected, outliers were excluded using the range test. Non-parametric analysis was used to calculate the conventional central 95% interval. Then the two-sided non-parametric 90% confidence interval of each percentile was determined for all dogs from group 3 and for all dogs from groups 3 and 4 (both $n > 120$). Finally, the effects of subgrouping (age, breed, sex, housing, intended use) in dogs older than 1 year were examined using the Kruskal–Wallis test and $p < 0.05$ was considered significant. All calculations were performed using statistical software (SAS Institute, Version 8.1, Cary, NC, USA).

## Results

Initially 314 dogs were included in the study. However, six had to be excluded, two because of severe lipaemia despite being fasted, two were very obese, and the remaining two had increased serum enzyme activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), alkaline phosphatase (AP), glutamate dehydrogenase (GLDH), and $\gamma$-glutamyl transferase (GGT) without historical or physical evidence of liver disease. For these last two dogs, the inclusion and exclusion criteria and the analytical procedures were controlled according to the IFCC recommendations (Solberg 1983). They were excluded from the study because the origin of these increases could not be found, and no other invasive diagnostic test could be performed. In five dogs serum sodium fluoride was not available. The activity of AP and that of GGT could not be measured in another four dogs. We repeated the analysis of these samples to exclude technical faults. Although the exact cause of the problem could not be identified, possibilities included a very low or absent activity of these enzymes, or the presence of inhibiting substances in the serum of these patients.

One ASAT value was determined to be a statistical outlier using the range test (Reed et al. 1971) and therefore excluded. All dogs were divided into nine breed groups, but two of them (groups 2 and 7) were too small ($n = 2$) and were excluded from the statistical variance analysis.

The results for the biochemical values are presented in Table 3. All estimated reference limits were in the 90% confidence interval, showing a good precision. Age significantly influenced 20 of the 22 values: only potassium and ASAT were not influenced by age. However, the effect of age was of clinical relevance only for 14 values. Clinical relevance was defined by a marked difference in the reference interval between the single subgroup and the whole population. This difference could actually influence clinical interpretation of the laboratory data. For some parameters a clearly significant difference of the median value was accompanied by only minimal differences in the reference intervals. An age-associated increase was found for total protein (TP) (Fig.1) as well as for albumin (Alb), total bilirubin,

**Table 2** Breed groups according to the F.C.I. nomenclature of dog breeds

| Breed groups | Breeds | Number of dogs |
|---|---|---|
| Group 1 (sheepdogs) | GSH, Tervueren, Canadian Sheepdog, Beauceron, Border Collie, Briard, Berger Picard, Malinois, Puli, Old English Sheepdog, Welsh Corgi, Collie | 87 |
| Group 2 (Schnauzer) | Giant Schnauzer | 5 |
| Group 3 (Molossian) | Saint Bernard Dog, Great Dane, Rottweiler,Newfoundland, Boxer, Hovawart | 22 |
| Group 4 (Swiss Mountain dogs) | Appenzell Cattledog, Entlebuch Cattledog, Great Swiss Mountain Dog, Bernese Mountain Dog | 22 |
| Group 5 (Terrier) | Border Terrier, German Hunting Terrier, Jack Russel Terrier, Manchester Terrier, Airedale Terrier, West Highland White Terrier, Norfolk Terrier, Staffordshire Terrier, Yorkshire Terrier | 13 |
| Group 6 (sled dogs) | Alaskan Malamute, Samoyed, Siberian Husky | 7 |
| Group 7 (Dalmatian) | Dalmatian | 15 |
| Group 8 (pointing dogs) | Weimaraner, Hungarian Short-haired Pointing Dog, German Wire-haired Pointing Dog, Griffon, Gordon Setter, Small Munsterlander | 8 |
| Group 9 (Retriever) | Flat Coated Retriever, Labrador Retriever, Golden Retriever, Nova Scotia Duck Tolling Retriever | 41 |

No group assignment – Australian Cattle Dogs, Doberman Pinscher, Dachshund, Hounds, Rhodesian Ridgebacks, English Cocker Spaniel, Sussex Spaniel, Havanese, Miniature Poodle, Toy Poodle, Standard Poodle, Tibetan Terrier, Tibetan Spaniel, Kromfohrlander, French Bulldog, Afghan Hound and Borzoi (Total 26)

**Table 3a** Reference values with clinically relevant age influences for all dogs of group 1 (0–0.5 years) and for all dogs of groups 3 and 4 (> 1 year)

| Value | Group | *n* | Median | Range | 95% Interval |
|---|---|---|---|---|---|
| GLUC (mmol/l) | 1 | 23 | 6.68 | 5.34–9.14 | 5.34–9.14 |
| GLUC (mmol/l) | 3 + 4 | 254 | 5.27 | 3.26–7.29 | 4.03–6.52 |
| TP (g/l) | 1 | 25 | 51.3 | 40.3–63.4 | 40.3–63.4 |
| TP (g/l) | 3 + 4 | 257 | 65.9 | 54.8–80.7 | 57.3–74.9 |
| ALB (g/l) | 1 | 25 | 30.4 | 22.6–34.3 | 22.6–34.3 |
| ALB (g/l) | 3 + 4 | 257 | 35.3 | 27.4–43.1 | 29.7–40.0 |
| Urea (mmol/l) | 1 | 25 | 5.15 | 3.07–8.23 | 3.07–8.23 |
| Urea (mmol/l) | 3 + 4 | 257 | 6.21 | 2.78–15.66 | 3.45–11.11 |
| CREA (µmol/l) | 1 | 25 | 41 | 24–78 | 24–78 |
| CREA (µmol/l) | 3 + 4 | 257 | 88 | 29–134 | 53–120 |
| BILI (µmol/l) | 1 | 25 | 1.0 | 0.2–2.3 | 0.2–2.3 |
| BILI (µmol/l) | 3 + 4 | 257 | 2.0 | 0.3–5.9 | 0.6–4.3 |
| Ca (mmol/l) | 1 | 25 | 2.95 | 2.59–3.29 | 2.59–3.29 |
| Ca (mmol/l) | 3 + 4 | 257 | 2.72 | 2.01–3.04 | 2.50–2.93 |
| P (mmol/l) | 1 | 25 | 3.13 | 1.91–4.13 | 1.91–4.13 |
| P (mmol/l) | 3 + 4 | 257 | 1.41 | 0.82–2.87 | 0.93–1.93 |
| Fe (µmol/l) | 1 | 25 | 24.3 | 6.5–43.2 | 6.5–43.2 |
| Fe (µmol/l) | 3 + 4 | 257 | 29.2 | 7.8–61.9 | 15.5–52.0 |
| ALAT (IU) | 1 | 25 | 24 | 15–90 | 15–90 |
| ALAT (IU) | 3 + 4 | 257 | 48 | 16–253 | 24–124 |
| AP (IU) | 1 | 25 | 153 | 85–224 | 85–224 |
| AP (IU) | 3 + 4 | 253 | 41 | 8–234 | 10–128 |
| CK (IU) | 1 | 25 | 416 | 145–1064 | 145–1064 |
| CK (IU) | 3 + 4 | 257 | 144 | 28–1516 | 64–390 |
| GGT (IU) | 1 | 25 | 3 | 1–4 | 1–4 |
| GGT (IU) | 3 + 4 | 256 | 4 | 1–9 | 1–7 |
| GLDH (IU) | 1 | 25 | 6 | 3–19 | 3–19 |
| GLDH (IU) | 3 + 4 | 257 | 4 | 1–22 | 2–10 |

**Table 3b** Reference values without clinically relevant age influences for all dogs of groups 3 and 4 (> 1 year)

| Value | *n* | Median | Range | 95% Interval |
|---|---|---|---|---|
| TRIGLYC (mmol/l) | 257 | 0.50 | 0.21–2.26 | 0.29–1.53 |
| CHOL (mmol/l) | 257 | 5.85 | 2.59–12.99 | 3.53–9.96 |
| Na (mmol/l) | 257 | 149 | 142–163 | 144–155 |
| K (mmol/l) | 257 | 4.7 | 3.6–5.6 | 4.1–5.3 |
| Cl (mmol/l) | 257 | 117 | 92–150 | 106–135 |
| AMYL (IU) | 257 | 624 | 249–1767 | 333–1262 |
| ASAT (IU) | 256 | 35 | 16–96 | 20–73 |
| LIP (IU) | 257 | 96 | 0–2208 | 10–1329 |

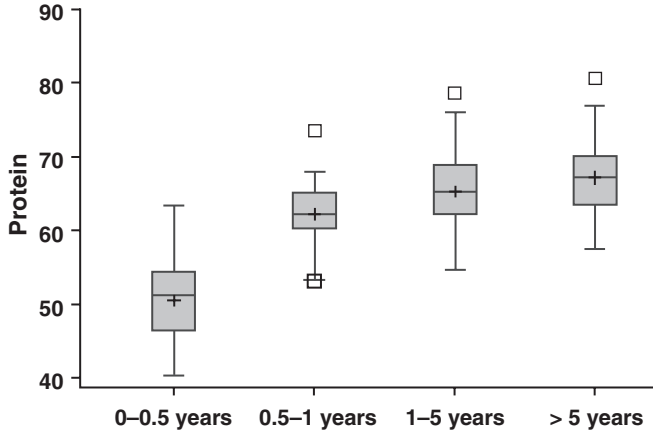


**Fig. 1** Age-associated increase of total protein (g/l). The horizontal limits of the box define the upper and lower quartiles enclosing the central 50% of the observations, with the median marked by a horizontal line within the box and the mean by a cross. The whiskers are vertical lines extending from the box as the minimum and maximum values of the set of observations

creatinine (Crea), urea, ALT, GGT and iron. An age-associated decrease was noticeable for phosphorus (Fig. 2) as well as for glucose, calcium, AP, GLDH and creatine kinase (CK).

Significant breed effects (Table 4) could be found for 10 of the 22 parameters: TP, Crea, total bilirubin, potassium, calcium, amylase, AP, GGT, lipase. The following were clinically relevant: lower total protein concentrations for retrievers (group 9), lower total bilirubin concentrations for terriers (group 5), higher total bilirubin for Molossians (group 3), and lower lipase activity for sled dogs (group 6).

Significant differences between males and females were present for six of the 22 variables but had no clinical relevance. Housing and intended use each influenced four of the values, but these differences were of no clinical significance. There were no statistically significant influences ($p > 0.05$) due to age, sex, breed, housing or intended use for the ASAT activity.

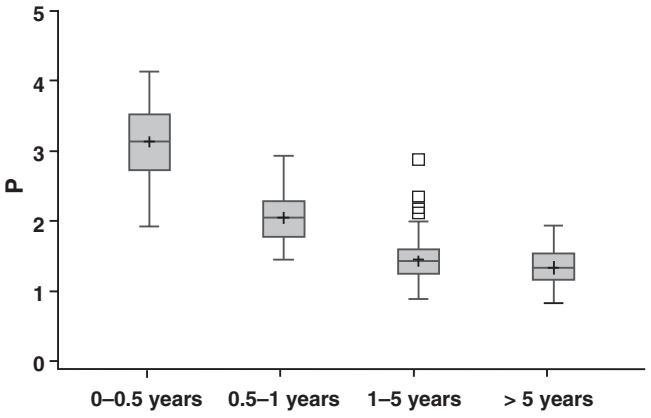


**Fig. 2** Age-associated decrease of phosphorus (mmol/l). The horizontal limits of the box define the upper and lower quartiles enclosing the central 50% of the observations, with the median marked by a horizontal line within the box and the mean by a cross. The whiskers are vertical lines extending from the box as the minimum and maximum values of the set of observation

## Discussion

Comparison with reference values derived from the literature is difficult because of regional biological variations and breed influences. Furthermore, the use of different analytical and statistical methods can also have an effect on the reference values. This is especially true for enzyme activities estimated at different temperatures, as was demonstrated by Keller (1986).

### Reference values

We compared our reference values for all dogs >1 year ($n = 257$) with the data from three other publications (Jacobs et al. 2000; Weskamp 1994; Dereser 1990). A good correlation was present for most of the organic substances and the electrolytes. Our upper reference limits for ALAT, ASAT, AP and lipase were higher than those determined by Jacobs et al. (2000). This difference could be due to biological, geographical and breed variations between Canada and Switzerland, or possibly to intraindividual variations. Different studies in humans (Costongs et al. 1985) and dogs (Jensen and Aaes 1993; Leissing et al. 1985) found that enzymes may show a high intraindividual variation. Furthermore, studies in humans showed that population-based reference values were not sensitive enough to demonstrate differences in concentrations of blood values over a time period for most persons, even if they were subgrouped for age or sex (Costongs et al. 1985; Solberg 1995). As mentioned earlier, the enzyme activities measured at 25°C in another study (Dereser 1990) were up to 50% lower than our results and those of Jacobs et al. (2000), where the analytical temperature was 37°C.

### Influence of age

Age tended to decrease the serum glucose concentration, as previously reported by others (Weskamp 1994; Kaspar and Norris 1977). The presence of higher calcium and phosphorus concentrations in young dogs attributed to skeletal growth (Broulet et al. 1986; Kuhl et al. 2000) was confirmed, as well as decreasing serum AP activities with age (Broulet et al. 1986; Keller and Wall 1982), where the skeletal isoenzyme is responsible for the increased AP activity in growing dogs (Rogers 1976; Sanecki et al. 1993). Our data also confirm previous reports stating that CK activity is higher in young dogs, and decreases when the animals reach adulthood (Kaspar and Norris 1977; Kraft et al. 1995; Keller and Wall 1982). As well as the different cell metabolism of growing individuals (Keller 1986), stress and high physical

**Table 4** Parameters showing clinically relevant breed effects

| Value | Breed group | *n* | Median | Range | 95% Interval |
|---|---|---|---|---|---|
| TP (g/l) | 1 (sheepdogs) | 80 | 66.7 | 55.6–77.0 | 58.2–74.5 |
| TP (g/l) | 3 (Molossian) | 21 | 67.6 | 57.3–72.8 | 57.3–72.8 |
| TP (g/l) | 4 (Swiss Mountain dogs) | 17 | 65.7 | 58.3–71.7 | 58.3–71.7 |
| TP (g/l) | 5 (Terrier) | 9 | 68.7 | 64.0–73.5 | 64.0–73.5 |
| TP (g/l) | 6 (sled dogs) | 7 | 66.8 | 62.0–70.2 | 62.0–70.2 |
| TP (g/l) | 8 (Dalmatian) | 7 | 66.7 | 57.7–73.7 | 57.7–73.7 |
| TP (g/l) | 9 (Retriever) | 37 | 61.3 | 55.3–71.0 | 55.3–71.0 |
| BILI (μmol/l) | 1 (sheepdogs) | 80 | 2.0 | 0.3–4.6 | 0.6–4.1 |
| BILI (μmol/l) | 3 (Molossian) | 21 | 2.4 | 0.9–5.9 | 0.9–5.9 |
| BILI (μmol/l) | 4 (Swiss Mountain dogs) | 17 | 2.1 | 1.1–3.4 | 1.1–3.4 |
| BILI (μmol/l) | 5 (Terrier) | 9 | 1.6 | 0.6–2.4 | 0.6–2.4 |
| BILI (μmol/l) | 6 (sled dogs) | 7 | 2.4 | 1.2–3.5 | 1.2–3.5 |
| BILI (μmol/l) | 8 (Dalmatian) | 7 | 2.6 | 0.3–4.8 | 0.3–4.8 |
| BILI (μmol/l) | 9 (Retriever) | 37 | 2.1 | 0.9–4.6 | 0.9–4.6 |
| LIP (IU) | 1 (sheepdogs) | 80 | 88 | 3–1329 | 7–769 |
| LIP (IU) | 3 (Molossian) | 21 | 162 | 29–1062 | 29–1062 |
| LIP (IU) | 4 (Swiss Mountain dogs) | 17 | 161 | 64–2208 | 64–2208 |
| LIP (IU) | 5 (Terrier) | 9 | 55 | 23–338 | 23–338 |
| LIP (IU) | 6 (sled dogs) | 7 | 27 | 4–59 | 4–59 |
| LIP (IU) | 8 (Dalmatian) | 7 | 202 | 41–860 | 41–860 |
| LIP (IU) | 9 (Retriever) | 37 | 46 | 0–584 | 0–584 |

activity may also influence enzyme activities in dogs (Ilkiw et al. 1989; Matwichuk et al. 1999). The age-associated increase of total protein and albumin has also been described by others (Kaspar and Norris 1977; Kraft et al. 1996). This increase seems attributable not only to an increasing immune stimulation resulting in an elevated globulin fraction (Yale and Balish 1976), but also to an increasing albumin production, probably resulting from better liver function and intestinal absorption (Wolford et al. 1988).

Our dogs under 6 months of age had the lowest concentrations of urea. This observation was also made by others (Kuhl et al. 2000) and thought to be caused by fasting and dietary factors (Chandler 1992). Alternatively, increased protein synthesis under the influence of growth hormone could also decrease the production of urea (Poffenbarger et al. 1990). Other possible explanations include increased metabolic state with increased glomerular filtration rate (GFR), and underdeveloped liver function. The decreased concentration of creatinine we observed in young dogs probably correlates with the smaller body size and muscle mass (Chandler 1992; Kuhl et al. 2000; Kraft et al. 1996; DiBartola 2000). However, other authors did not find age differences for creatinine (Abel and Schneider 1973; Strasser et al. 1997), possibly because they used the insensitive Jaffé method to estimate the creatinine concentration.

An age-associated increase for total bilirubin had also been previously reported (Feller 1983; Dereser 1990; Kraft et al. 1996). This phenomenon was thought to be due to increasing haemoglobin concentrations. This hypothesis was supported by the fact that greyhounds have higher reference values for both haemoglobin and total bilirubin than other breeds (Lassen et al. 1986; Steiss et al. 2000). Low concentrations of iron were found in our dogs under 6 months of age, confirming the results of a previous study (Passing and Brunk 1981). Most animals have low iron concentrations until 6 weeks after birth (Smith 1992). However, it is not clearly understood whether puppies experience an absolute or only a relative iron deficiency. The latter could be due to iron storage in the reticuloendothelial system (RES) or to higher concentrations of acute-phase proteins. Two other publications did not report lower iron concentrations in puppies (Dereser 1990; Sehr 1986). We found lower ALAT and GGT activities in our puppies, as had been previously reported (Kaspar and Norris 1977; Kuhl et al. 2000, Dereser 1990; Keller and Wall 1982). These changes in plasma enzyme activities are a sign of cell growth, adaptation and differentiation of the organs and metabolism (Keller 1986).

### Influences of breed

Our finding of lower total protein concentrations in retrievers was also reported in other publications (Kuhl et al. 2000; Matwichuk et al. 1999; Skerritt and Jenkins 1986). Although the numbers of dogs in the various breed groups are small, and differences between the reference values are small, this finding could reflect some difference in protein metabolism specific to retrievers. However, further studies with larger numbers of dogs are needed to investigate the relevance of this phenomenon in more detail. The range of serum lipase activity is broader in groups 3 (Molossians) and 4 (Swiss Mountain dogs) than in group 6 (sled dogs). This may be due to the many different origins of this enzyme. Lipase activity can be elevated following pancreatic diseases, enteritis, renal diseases and glucocorticoid administration (Meyer and Harvey 1998), and we cannot exclude the fact that some dogs may have been subclinically affected by such disorders. Furthermore, the narrower reference range seen in sled dogs could reflect the homogeneity of this breed group consisting of two breeds, with most dogs being kept outside and used for a single purpose. A decreased amylase activity was previously described in greyhounds during the racing period (Lassen et al. 1986). No reason could be found to explain this phenomenon. The reason for breed differences in serum bilirubin concentration is not known, but this finding has not been reported before. However, because of the small numbers of dogs in some breed groups, the relevance of these differences need to be confirmed in further studies including larger numbers of dogs in each specific breed group.

In conclusion, we successfully established canine clinical chemistry reference values for the Hitachi 912. The IFCC Recommendations on Reference Values offered a good framework for establishing standardised reference values. Using the IFCC standard concept for determining reference values in veterinary medicine would make it possible for several laboratories to share the same reference values. The analytical temperature of enzyme activities is important in comparing one's own reference values with the literature because of the large differences between measurements at 37°C and at 25°C. Differences due to sex, housing and intended use were not clinically relevant. However, our results clearly indicate that subgrouping according to age and breed is important to obtain accurate reference values. Further studies are necessary to investigate the breed-related differences we identified.

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